The effect of auxiliary substances on pharmaceutical availability of medicinal substances contained in dry extract from small-flowered willow herb (*Epilobium parviflorum* Schreb.)

Z. MARCZYŃSKI, M. ZGODA

Department of Pharmacy and Applied Pharmacy, Medical University of Łódź, Muszyńskiego 1, 90-151 Łódź, Poland

Summary

Therapeutic agents of natural origin, administered in prostatic disorders, have wider experimental and clinical documentation than the synthetic medicines, fashionable, but relatively new on the market. Their superiority is based first of all on versatile points of effect, almost total lack of side effects and a significantly low cost of therapy. Bearing in mind the above mentioned, an attempt was made to produce an oral solid form of a drug from dry aqueous extract of small-flowered willow herbs (*E. parviflorum*), using some formulating components of variable adsorptive properties.

Taking into account the composition and granulometric properties of the extract, two alternative technological processes have been suggested to produce oral solid model pharmaceutical agents.

These model forms were subjected to the following morphological tests determining: the appearance of tablets, their mass, friability and effective disintegration time. Furthermore, pharmaceutical availability of the model form of the drug was investigated. The effect of formulating components on the course of quantitative determination of active agents was assessed in the tablets.

Key words: Epilobium parviflorum Schreb., auxiliary substances, pharmaceutical availability

The therapeutic agents currently used in benign prostatic hypertrophy may be divided into: α-adrenergic-receptor blocking agents, hormonal drugs, polyene macrolides and plant drugs [1-4].

The aqueous extracts from *E. parviflorum* have been successfully used in folk medicine for many years in the treatment of bladder, kidney and prostatic pathological states. The phytochemical and pharmacological investigations carried out

so far fully justify the application of *E. parviflorum* and other species of *Epilobium* in the treatment of these disorders. They have demonstrated that the aqueous extracts from those plants are active [5, 6].

Aqueous extracts from *E. parviflorum* show diuretic, antibacterial, antineoplastic, immunostimulating and anti-inflammatory effects, and they inhibit 5-α-reductase and aromatase activity, as well as stimulate the defensive function of granulocytes [7-11]. The application of cytometric analysis demonstrates that extracts from various species of *Epilobium* inhibit the progress of cellular cycle in the phase G0/G1 [12]. The significant therapeutic value, lack of side effects or interaction of this material are factors that encourage to introduce a solid form of therapeutic agents with dry extract from *E. parviflorum* into effective pharmacotherapy. The aim of this study was to obtain the model oral solid form of a drug containing an extract from *E. parviflorum*, using formulating components of variable adsorptive properties. Taking into consideration the physicochemical properties of the dry extract, two alternative technological processes were worked out to produce a model oral solid form of the drug containing 250 mg of dry extract from *E. parviflorum* [13, 14]. Investigating pharmaceutical availability of this drug, an attempt was made to estimate not only the ways of formulating but also the effect of the components on the adsorption of this therapeutic agent in the process of the tablet disintegration. Furthermore, the effect of formulating components on the course of quantitative determination of the therapeutic agents was estimated in this form of a drug. The results of the carried out investigations are subject of this research work.

MATERIAL AND METHODS

The study was carried out using: dry aqueous extract from *E. parviflorum* (Phytopharm, Klęka S.A.), ethyl alcohol (Polmos), microcrystalline cellulose (Serva Feinbiochemia), Kollidon 25 (Fluka AG), lactose, potato and corn starch purchased from Terpol (Sieradz, Poland), Vivapur 12 (Fluka AG), talc (Biocom), magnesium stearate, potassium hydrogen phosphate, sodium hydroxide and 0.1 mol/l HCL purchased from "POCh" (Gliwice, Poland).

Chromatoplates:

1. DC – Plastikfolien, Cellulose F254
2. DC – Alufolien, Kieselgel 60
3. DC – Alufolien, Kieselgel 60 F254
4. DC – Fertigplatten, Kieselgel 60 F254

TECHNOLOGY

Taking into consideration requirements associated with the availability of therapeutic components found in an oral solid form of the drug, the composition of

auxiliary substances has been suggested (Table 1) to produce a model form of the pharmaceutical agent, an uncoated tablet [15, 16].

Table 1.

The share of formulating components and dry extract from *E. parviflorum* in produced model tablets.

formulating components	series 1	series 2	series 3	series 4	series 5	series 6	series 7	series 8	series 9	series 10
dry extract from *E. parviflorum*	+	+	+	+	+	+	+	+	+	+
lactose	+	+	+	+						
starch	+	+	+			+		+	+	+
Vivapur 12			+	+	+		+	+	+	+
magnesium stearate	+					+	+			
talc		+	+	+	+			+	+	+

All the formulating components were weighed for a series from 100 to 200 tablets. Dry extract from *E. parviflorum* and part of powdered auxiliary substances were mixed thoroughly and appropriate amount of ethanol was added and mixed to obtain granulated mass on the sieve of mesh diameter 0.75 mm. Next, the granulated mass was dried in air stationary circulation at room temperature for 24 hours, and then sieved again to separate dust. Ready granules were mixed with the remaining auxiliary substances including a lubricant. Having obtained stable and durable density, the mass was compressed into tablets with Korch–Erweka tableting machine, using flat stamps with a diameter of 12 mm.

Table 2 presents the way of manufacturing model tablets with dry extract from *E. parviflorum* and auxiliary substances enabling to produce a model pharmaceutical agent in the form of uncoated tablet with the method of direct compressing into tablets. These model tablets contain 250 mg of aqueous dry extract from *E. parviflorum*.

Table 2.

The share of formulating components and dry extract from *E. parviflorum* in produced model tablets.

composition of tablet mass	1 tablet	100 tablets	content, %
dry extract from *E. parviflorum*	250 mg	25.0 g	62.5
microcrystalline cellulose	+	+	31.25
corn starch	+	+	1.75
Kollidon 25	+	+	2.5
magnesium stearate	+	+	2.0

All the formulating components were weighed for a series containing at least 100 tablets. Dry extract from *E. parviflorum* and auxiliary substances were mixed thoroughly. Next the tablets were made by Korch–Erweka tableting machine, using flat stamps with a diameter of 12 mm.

The manufactured tablets were tested in order to determine their technological parameters conditioning their therapeutic usefulness. The tests included: evaluation of the appearance of the tablet (size), precision of dosage (determination of the tablet mass), testing the durability (friability) and effective disintegration time, as well as statistical evaluation of hardness. The measurements were performed in Research Laboratory "Terpol" Sieradz, using a TB-M Erweka apparatus.

Standard curves were determined for therapeutic agents in 0.1 M HCL and in phosphate buffer solution, pH 6.8. According to the requirements of FP VI, pharmaceutical availability was investigated *in vitro* in 0.1 M HCL and phosphate buffer, pH 6.8. These tests were performed in an apparatus testing therapeutic agent release by spatula method. Using Specord M 40, the content of the therapeutic substances was determined in the range 220–330 nm. Furthermore, a chromatographic analysis of dry extracts contained in the model form of a drug was carried out. The receiver fluids were used as developing systems to investigate pharmaceutical availability.

RESULTS AND DISCUSSION

Taking into consideration physicochemical properties of dry aqueous extract from *E. parviflorum*, an attempt has been made to produce an oral solid form of the drug in the population dose of 250 mg of the dry extract.

Two alternative technological processes were suggested. (1) Direct compressing of the granulated mass mixed with polar solvents into tablets. However, the tablets manufactured in this way did not have the required mass (400 mg) due to insufficient powder density (high degree of fluffiness). (2) Pharmacopeal ethanol was used to increase the powder density, which did not affect the quantitative composition of the extract, while at the same time enabled obtaining comparable powder density of the extract with the used auxiliary substances, and in consequence the alternative technological process could be applied in the form of direct compressing into tablets. The results of morphological tests of the model form of a drug are shown in Table 3.

A statistical analysis of mean masses of the model tablets containing the extract with standard deviation in the range: dx (min) = 0.38%, +/- dx (max) = 2.47% demonstrated that they are within the limits of pharmacopeal standard. The obtained results prove the tablet mass to be homogeneous and the filling niche in the tableting machine over the bottom stamp to be symmetrical. This condition is reflected in the content of therapeutic agents in solid model form of a drug so that the declared dose of the therapeutic agent with calculated standard deviation guaran-

tees therapeutic effectiveness. However, in each case the therapeutic agent content distribution in a dose does not affect the principles of pharmacopeal correctness. We were not successful in producing tablets with dry extract from *E. parviflorum* with the disintegration time shorter than 15 minutes, the reason of prolonged disintegration time being lipophilic character of ballast bodies contained in dry extract of the plant.

Table 3.

The results of morphological tests of tablets containing dry extract from *E. parviflorum*.

morphological tests	series 1	series 2	series 3	series 4	series 5	series 6	series 7	series 8	series 9	series 10
area of tablet (sq mm)	124.80	128.79	130.40	129.01	129.97	120.47	126.43	125.62	129.34	128.74
time of disintegration (h/min/sec)	0/53/20	0/54/30	0/53/42	1/28/15	1/38/30	1/20/23	2/16/15	1/46/10	0/56/40	1/31/18
friability (%)	0.40	0.86	0.37	0.29	0.20	0.35	0.08	0.69	0.90	0.53
deviation from mean mass %	0.40	0.41	0.40	0.38	0.48	2.77	0.44	0.82	1.29	1.33
hardness (x +/- ts)	123 +/- 19.5	84 +/- 50.3	63 +/- 3.1	62.7 +/- 5.6	64.3 +/- 4.4	47.0 +/- 6.7	110.0 +/- 47.2	40.0 +/- 15.2	43.7 +/- 7.8	57.0 +/- 10.1

The kinetics of the therapeutic agents release from tablets in 0.1 M HCL is presented in Fig. 1 a and b.

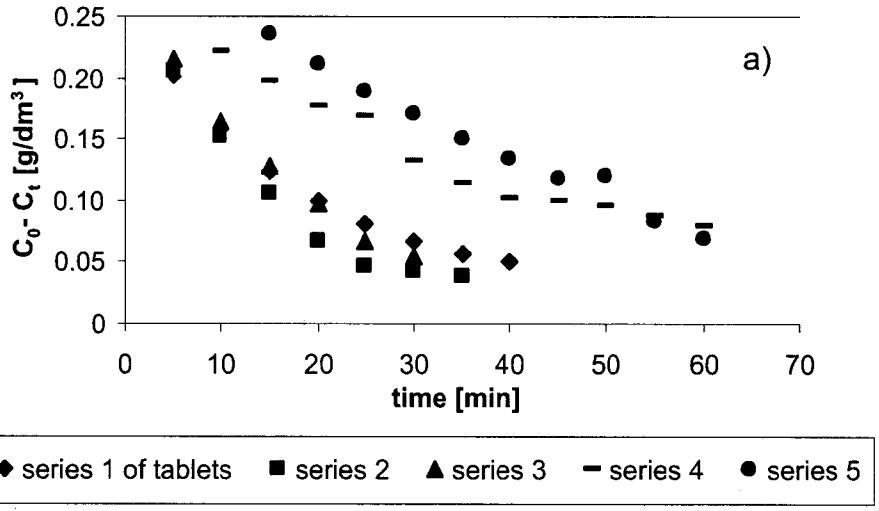

Fig. 1a. Kinetics of therapeutical agent's release from tablets.

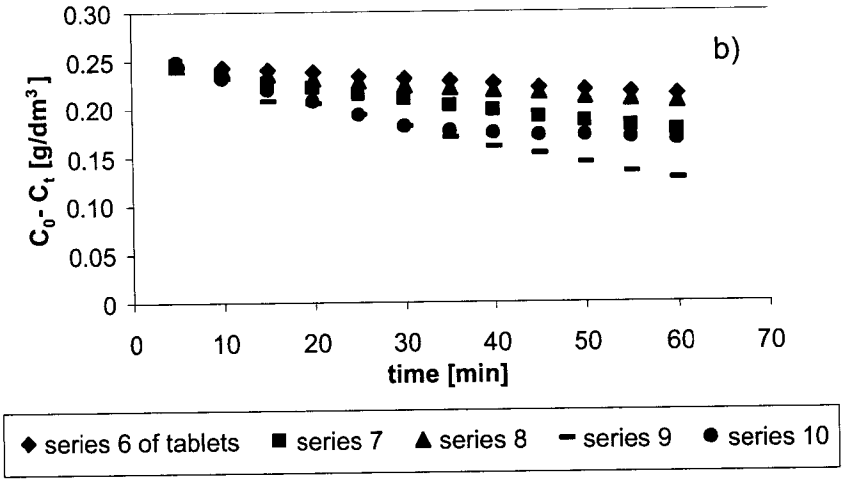

Fig. 1b. Kinetics of therapeutical agent's release from tablets.

The determined pharmaceutical availability of the therapeutic agent in the manufactured form of a drug is also the function of its hardness. A high coefficient of hardness prolongs the adsorption of water and thus the effective disintegration time. The optimal hardness of tablets along with low friability (predisposition to breaking) makes the packing of the drugs in blisters technologically easy (assures morphological durability of the preparation when it is shelled out of the blister niche).

The results of chromatography of dry plant extracts contained in the model form of a drug are shown in Table 4.

Table 4.

The results of chromatography with TLC method (thin-layer chromatography).

developing system	Rf		
	extract in aqueous environment	extract in 0.1 M HCl environment	extract in phosphate buffer, pH 6.8
0.1 M HCL	1*. 0.41; 0.86 2*. 0.43; 0.88 3*. 0.18; 0.93 4*. 0.80	1. 0.40; 0.87 2. 0.49; 0.89 3. 0.10; 0.92 4. 0.75	1. 0.87 2. 0.43; 0.77 3. 0.14; 0.76 4. 0.78
phosphate buffer, pH 6.8	1. 0.86 2. 0.91 3. 0.83 4. 0.88	1. 0.87 2. 0.85 3. 0.78 4. 0.85	1. 0.87 2. 0.76 3. 0.78 4. 0.87

* chromatographic plates
1. DC – Plastikfolien, Cellulose F_{254}; 2. DC – Alufolien, Kieselgel 60; 3. DC – Alufolien, Kieselgel 60 F_{254};
4. DC – Fertigplatten, Kieselgel 60 F_{254}.

The results presented in Table 4 demonstrate that distribution of flavonoids in 0.1 M HCl takes place in accordance with the rule of their pKa, whereas in a phosphate pH 6.8 buffer solution they are in an ionised form, which is proved by lower and comparable coefficient R*f* values. The results of chromatography confirm that there are flavonoids with determined solubility, or high coefficient of separation from plant material.

CONCLUSIONS

The obtained results indicate that it is possible to work out a technology of production of an oral solid form of a drug from dry aqueous extract from *E. parviflorum*. The major problem requiring further investigations is modification of the effective disintegration time, which can be obtained by changing the saccharides used in formulation. Our results are the base for further studies.

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WPŁYW SUBSTANCJI POMOCNICZYCH NA DOSTĘPNOŚĆ FARMACEUTYCZNĄ SUBSTANCJI LECZNICZYCH ZAWARTYCH W SUCHYM EKSTRAKCIE Z ZIELA WIERZBOWNICY DROBNOKWIATOWEJ (*EPILOBIUM PARVIFLORUM* SCHREB.)

Z. MARCZYŃSKI, M. ZGODA

Zakład Farmacji Aptecznej, Katedra Farmacji Stosowanej,
Wydział Farmaceutyczny, Uniwersytet Medyczny w Łodzi,
ul. Muszyńskiego 1, 90-151 Łódź

Streszczenie

Stosowane w schorzeniach prostaty środki naturalnego pochodzenia mają lepszą dokumentację doświadczalną i kliniczną niż modne, lecz stosunkowo krótko znajdujące się na rynku leki syntetyczne. Ich przewaga wynika przede wszystkim z wielofunkcyjnych punktów uchwytu, prawie całkowitego braku działania ubocznego oraz znacznie niższych kosztów terapii. Biorąc powyższe po uwagę, podjęto próbę otrzymania stałej doustnej postaci leku zawierającej suchy wodny wyciąg z ziela wierzbownicy drobnokwiatowej (*Epilobium parviflorum* Schreb.) przy użyciu składowych formulacyjnych o zmiennych właściwościach adsorpcyjnych. Mając na uwadze skład i właściwości granulometryczne wyciągu zaproponowano dwa alternatywne procesy technologiczne pozwalające otrzymać stałe, modelowe, doustne środki farmaceutyczne.
Wytworzone modelowe postacie leku poddano badaniom morfologicznym, stosując następujące kryteria: ocena wyglądu tabletek, określenie masy, ścieralności i efektywnego czasu rozpadu. Wykonano ponadto badania dostępności farmaceutycznej środka leczniczego z otrzymanej modelowej postaci leku. Dokonano oceny wpływu składowych formulacyjnych na przebieg ilościowego oznaczania substancji czynnych w tabletkach.

Słowa kluczowe: *Epilobium parviflorum Schreb., substancje pomocnicze, dostępność farmaceutyczna*